PLANT EXTRACTS

Technical field of the invention

The present invention relates to plant extracts that comprise plant secondary metabolites. In particular, the present invention relates to plant extracts that comprise saponins and/or their aglycone forms as well as methods of production thereof.

Background of the invention

Plants produce a vast and diverse assortment of organic compounds, the great majority of which do not appear to participate directly in growth and development. These substances, traditionally referred to as secondary metabolites or plant natural products, often are distributed among limited taxonomic groups within the plant kingdom. The functions of secondary metabolites remain largely unknown, although a number of compounds have been associated with e.g. protection against herbivores and protection against microbial infection, as attractants for pollinators and seed-dispersing animals, and as compounds that influence competition among plant species (allelochemicals).

There is a growing interest in plant natural products, since these products often have a wide range of applications in different kinds of industries, including pharmaceutical industries, cosmetic industries, food industries, detergent industries, etc.

Saponin is an example of a group of plant secondary metabolites. Saponins are glycosylated compounds classified as either triterpenoids, steroids, or steroidal glycoalkaloids. Saponins consist of one or two sugar moieties which are coupled to the aglycon (mono- and bisdesmosides, respecitvely).

Saponins can be hydrolysed to sapogenins and sugar moieties by acid hydrolysis or enzymatic methods. Saponins are water soluble high molecular weight compounds.

Saponins have a wide range of applications. Saponins have the ability of lowering surface tension and the word "saponin" also reflects this as "sapo" is the latin word for soap. Saponins therefore have potential applications in the cosmetic and in the detergent industries. Saponins furthermore have the ability of forming insoluble complexes with cholesterol, which makes some of them suitable for use in the pharmaceutical industry as cholesterol lowering agents. Saponins also have other therapeutical effects. Saponins from chestnut do e.g. possess anti-inflammatory characteristics. Saponins, when injected into the blood stream, are furthermore highly toxic due to their haemolytic properties. Saponins are usually relatively harmless when ingested orally. Steroidal saponins are of great interest owing to their relationship with such compounds as the sex hormones, cortisone, diuretic steroids, vitamin D and cardiac glycosides. Also, saponins are associated with formation of immunostimulating complexes (ISCOMs) (Morein et al., (1995) Clinical Immunotherapeutics 3: 461-475) that are useful in vaccine strategies.

At present however, a major obstacle in exploiting the wide range of potential applications of saponins is the fact that commercially available saponins are relatively expensive.

Commercially available plant extracts containing saponins are e.g extracts of *Saponaria officinalis, Quillaia* bark and stem, *Castanea sativa* seeds, and extracts of various *Yucca* species. Liquorice root, primula root, and senega root can also serve as raw material for saponin extracts. A problem in this field is that the available sources of saponin extracts are relatively few. And in some cases, e.g. *Quillaia* bark, the plants are often sparse and expensive

because they cannot be cultivated in an efficient manner. Usually the saponins are present in relatively low concentrations. The commercially available saponin extracts are thus often expensive and/or sparse. It should also be noted that the saponins are quite complicated compounds and it has not so far been profitable to develop methods for chemical synthesis of these compounds.

Plant extracts containing saponins and sapogenins are thus of general interest within a wide range of different industries. There is therefore a growing need in the art for alternative sources of saponin extracts and these plant sources should preferably be cheap, easy to obtain, and preferably the saponin content should be relatively high.

For the general description of saponins, extraction, production, and use of saponins reference is made to Ullman's Encyclopedia of Industrial Chemistry (1993), Vol. A23, pp. 485-498. Furthermore, the literature is abundant concerning the extraction, composition and specific effects of the individual saponins derived from plant materials.

The fruits from the butter trees; the African shea tree (*(Bassia) Butyrospermum parkii* or *Vitellaria paradoxa*) and Indian butter tree (*Bassia latifolia* and *B. longifolia*; or *Madhuca Sp.*) of the *Sapotacea* family contain seeds (nuts) that are suitable for extraction and production of butter fat. Shea butter fat and Indian butter fat have a wide range of applications including as food, food ingredient, emulsifier, and also as an ingredient in the production of cosmetics.

Extraction and production of butter fat leaves a press cake or an extracted residue (meal) as a by-product (waste product). It has proven difficult to find normal outlets for this by-product as an ingredient in e.g. animal feed. This is probably due to a content of secondary metabolites that are toxic to

mammals. Consequently the material is presently disposed off by e.g. burning.

An investigation of an alcohol extract from "mowrah meal" from the Indian butter tree *Bassia latifolia* as well as other related species revealed presence of a novel triterpene sapogenin called "bassic acid" ($C_{30}H_{46}O_5$) (Heywood et al., J. Chem. Soc. (1939), Part V, pp. 1124-1129). Bassic acid from *B. butryrace* was extracted with a solution containing 5% hydrochloric acid. Bassic acid was also identified in the by-product from shea tree. It is disclosed that saponins and sapogenins can be extracted from by-products by water or alcohol extraction. The document does not disclose any methods of stabilising the saponins in the watery solution from undergoing hydrolysis. Likewise, no possible applications of the extracts are disclosed herein and consequently no companies have been encouraged to exploit by-products for production of saponin/sapogenin extracts on basis of this study published in 1939.

Object of invention

One object of the present invention is to find a useful application, other than e.g. burning, of by-products from the production of shea butter fat and mowrah fat.

It is furthermore an object to provide methods of producing low-cost and high-quality saponin/sapogenin containing extracts. It is of especially importance to provide a chemically stable environment during manufacture and storage of the extract. And also to provide an environmentally friendly production method of said extracts.

Another object is to identify applications of butter tree extracts according to the invention.

Summary of the invention

The object of the present invention is achieved by a method of preparing an aqueous extract comprising saponins on basis of waste product from a butter tree of the *Sapotacea* family, said method comprising the following steps:

(i) mixing one part waste product with 4-30 parts of water;

(ii) incubating the mixture formed in step (i) under alkaline conditions; and

(iii) recovering an aqueous extract comprising saponins by removing solids from the alkaline mixture formed in step (ii).

The invention further relates to various uses of such extracts as well as to extracts prepared by the methods herein.

Definitions

"Butter tree": As used herein butter tree can be a shea butter tree, preferably *(Bassia) Butyrospermum parkii*, or an Indian butter tree (preferably *Bassia latifolia* or *B. longifolia;* sometimes referred to as *Madhuca sp.*) of the *Sapotacea* family.

"By-product": As used herein a by-product refers to the by-product from production of butter fat from shea butter tree or Indian butter tree. The terms "by-product", "waste-product", "butter cake meal", "press cake", "extracted residue", etc. are used interchangeably. The by-product may be virtually fat free or there may be considerable amounts of residual fat depending on the method of extraction.

"Saponins": Saponins are glycosylated compounds found in many plants classified as either triterpenoids, steroids, or steroidal glycoalkaloids. Saponins consist of one or two sugar moieties which are coupled to the

aglycon. Saponins can be hydrolysed to sapogenins and the corresponding sugar moieties by acid hydrolysis or enzymatic methods

"Water": Water according to the present invention refers to any aqueous solution, preferably in the form of pure or essentially pure water such as e.g. distilled water or tap water. Water can also refer to an aqueous solution comprising an alkaline substance or an alkaline buffer.

"Alkaline conditions": Extraction of butter tree waste product according to the present invention is carried out under alkaline conditions, i.e. the pH-value during the extraction process must be at least 7. The alkali constituent can be in the form of an alkaline substance or an alkaline buffer. The alkali constituent can be added to the water prior to mixture with butter tree waste product or it can be added after water has been mixed with the waste product. The alkali can be any substance with the ability to raise pH to a level of above 7. Examples of alkali substances include but are not limited to: NaOH, KOH, $Ca(OH)_2$, LiOH, NH_3, $Mg(OH)_2$, ammonium hydroxide, potassium-, sodium-, calcium-, ammonium-, or hydroxide carbonate.

Detailed description of the invention

In the present invention it is disclosed that water based plant extract from butter tree by-product comprises saponins and/or sapogenins and have a wide range of applications. Methods of production of such extracts are furthermore disclosed herein.

In abovementioned applications the extract may be purified and have a content of saponins and/or sapogenins or their derivatives in a concentration of min. 5% by weight calculated as sapogenins. This is especially the case in pharmaceutical as well as food applications and in a variety of cosmetic products. But in some applications there is no need for any special

purification of the extract e.g. agricultural use. In some cases it is even beneficial that the extract has its normal content of tannins, e.g. in. special skin care products or in leather tanning.

The normal procedure for extracting saponins and sapogenins involves use of an organic solvent such as e.g. alcohol or an alcohol/water. The methods disclosed in the present application employ water based extraction methods that are both efficient and environmentally friendly.

The evaporation of water can take place at atmospheric or reduced pressure, by spray drying or any standard processing method known by people skilled in the art.

The extract obtained so far is composed of a diversity of water soluble types of constituents. One group is the tannins. The tannins are a group of simple and complex phenol, polyphenol and flavonoid compounds, bound with starches, and often they are just classified as tannins simply as they contain variations on gallic acid. One simple way of fractionating the constituents is by applying ultra filtration to separate the constituents according to their molecular size.

Extracts obtained by the methods of the invention may be further subjected to a hydrolysing step converting a fraction of the saponins to their corresponding sapogenins. The sapogenin part may be further purified by recrystallisation and/or derivatised with fatty moieties to make it soluble in e.g. oil.

In a preferred embodiment of the invention the plant extract obtained by the process from *Butyrospermum parkii* or *Bassia sp.* is further characterised by a content of saponin and/or sapogenin or their derivatives of min. 5% by weight calculated as sapogenin.

In a first aspect of the present invention a method is disclosed wherein an aqueous saponin extract from a butter tree waste product is produced, said method comprising the following steps:

(i) mixing one part waste product with 4-30 parts of water;
(ii) incubating the mixture formed in step (i) under alkaline conditions; and
(iii) recovering an aqueous extract comprising saponin by removing solids from the alkaline mixture formed in step (ii).

The aqueous extraction is carried out by mixing water with waste product in step (i). One part of waste product is preferably mixed with 7-25 parts of water, more preferably 10-20 parts of water, and even more preferably 15-20 parts of water.

The alkali conditions in step (ii) are obtained by addition of an alkali in the form of a base and/or buffer. Addition of the alkali component raises the pH to a level of between 7-14, preferably 7-10, more preferably 7-9 and most preferably 7-8.

Incubation in step (ii) can be performed at any temperature of between 15 and 95 °C, e.g. at 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95 °C at a period of between 5 minutes and 5 hours, e.g. 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours.

Separation of solid components in step (iii) can take place by filtration or centrifugation. Examples of suitable filters include: plate, frame, and vacuum filters, that are preferably precoated with e.g. diatomaceous earth.

In order to ensure keepability one or more preservative agents such as benzoates or sorbates may be added.

Furthermore, the extract obtained in step (iii) can be further concentrated and/or purified by evaporation of water and/or by ultrafiltration and/or by recrystallisation.

In a preferred embodiment, an extract obtained by means of the methods disclosed herein is subsequently enriched with sapogenins by subjecting the extract to partly or complete hydrolysis, by means of acids or enzymes and thereby obtaining an extract enriched with sapogenins. The sapogenins may be further purified by recrystallisation. Furthermore, the saponins and/or the sapogenins of an extract according to the invention can also be chemically modified by e.g. derivatising with fatty moieties in order to increase oil solubility.

Extracts according to the present invention do have a number of different applications:

- as a food additive and/or food ingredient;
- as an ingredient in a detergent product;
- as an ingredient in a cosmetic product;
- as an active component in a pharmaceutical product for topical application;
- as an active component in a pharmaceutical product for lowering the level of serum cholesterol in a human being or in other mammals;
- as an active compound in a pharmaceutical product for treatment of inflammatory diseases;
- as an active compound in a pharmaceutical product for systemic administration, e.g. treatment of cardiac disorders or diuretic disorders, or vitamin D associated disorders;
- as an active component in the manufacture of a nutritional supplement;
- as an ingredient in immunostimulatory complexes (ISCOMs).

The plant extract e.g. may be used as a wetting agent or emulsifier. The extract can be used as a wetting agent in many applications e.g. spraying of pesticides and herbicides, dust control, etc. Furthermore it can be formulated with other surfactants, builders and ingredients normally used in detergents.

As to acceptable cosmetic ingredients, reference is made to handbooks covering geographical areas e.g. International Cosmetic Ingredient Directionary and Handbook published by The Cosmetic, Toiletry, and Fragrance Association. An acceptable pharmaceutical ingredient is to be understood as the ingredients and exipients monographed in any national or regional pharmacopoeia e.g. European Pharmacopoeia.

As for the food products, reference is made to the WHO/FAO listing of acceptable animal and plant derived ingredients.

The invention is illustrated in the following non-limiting examples:

Examples

EXAMPLE 1

Use of a shea extract as a wetting agent and emulsifier

1.1 Production of the extract

300 g defatted shea meal by-product was mixed with 6000 ml tap water. pH of this meal solution is neutral. pH was then elevated by addition of 6 g sodium carbonate (2 weight-% of the meal). Without addition of alkaline, the saponins will gradually hydrolyse and pH of the solution will also gradually decrease. The extract was then incubated at 80°C for 15 minutes and subsequently filtered. The resulting brown coloured extract had a content of

dry matter of 2.1% by weight. The extract was diluted to a content of 2% dry solid matter 0.02% Kathon CG (preservative) was added. The extract is named SHEX-2 in the following.

1.2. Surface tension properties of SHEX-2

Table 1: Surface tension at different concentrations

Parameter	Concentration in g/l	Surface tension at 22°C in dyn/cm
SHEX-2, undiluted	20	49.1
SHEX-2, 1:10	2	56.1
SHEX-2, 1:100	0.2	60.7
SHEX-2, 1:1000	0.02	72.0
Distilled water	0	72.4

The measured surface tensions illustrate that the crude extract functions as a wetting agent and surfactant at concentrations of dry solid matter of 0.2 – 20 g/l.

1.3 Interfacial tension

The interfacial tension between soya oil and SHEX-2, 1:10 (2 g/l) was < 5 dyn/cm. This illustrates the emulsifying properties of the extract.

1.4 Use of SHEX-2 as a defatting agent

A test person with hands oiled by normal salad oil rubbed 10ml. of SHEX – 2 in. By rinsing with water an emulsion was formed and after drying no traces of oil was left on the hands.

EXAMPLE 2

Use of a shea extract as a cosmetic ingredient

SHEX-2 was tested in the following shampoo formulation:

SHEX-2	25.0%
Nyfamid KDK (Cocoa fatty acid diethanol amide)	5.5%
Guardan 178 (Guar gum)	1.3%
Drom 7699 (Fragrance)	0.5%
Demineralised water	ad. 100.0%

A test of abovementioned formulation showed that SHEX-2 is suited as a cosmetic ingredient in a hair shampoo in combination with other normal cosmetic ingredients.

EXAMPLE 3

Use of a purified and concentrated shea extract as an ingredient in a therapeutic skin tonic.

3.1 Data profile for SHEX-14:

The crude extract from example 1 was further purified and concentrated to form an extract named SHEX-14. The product had the following properties:

Appearance:	Clear, yellowish liquid
Dry solid content:	14% by weight
Specific gravity (20°C):	1.055 g/ml
Surface tension (20°C):	46.5 dyn/cm

3.2 Composition of the skin tonic:

SHEX-14 was formulated into the following skin tonic product:

SHEX-14	50%
Glycerine B.P.	15%
Demineralised water	35%

3.3 Test on atopic skin

The product was tested on skin areas with atopic eczema. The product was applied once daily on damp skin after showering. After one week there was a clear improvement of the eczema; reduced scaling and a reduction of inflammatory redness.

EXAMPLE 4

Use of a concentrated and purified shea extract as an ingredient in a food product

SHEX-14 was used as a foam stabiliser in a concentrated soft drink. "Fruiss Grenadine" (Rutin s.a., France) was selected as the test soft drink as it is formulated without foam additives and has no ability to foam. The concentrated soft drink was added varying amounts of SHEX-14. The samples were diluted with water (1:9) to normal use concentration and transferred to glass cylinders fitted with glass stoppers. After shaking the cylinders they were left for observation of the foam stability. At a concentration of SHEX-14 of 56 g dry matter pr. 100 litre of soft drink the creamy foam ring had a stability of more than 30 minutes.

EXAMPLE 5

Yield of shea extract at different meal/water ratios

The following table show the yield of dry matter in the shea extract at extractions performed at different meal/water ratios. The extractions were performed at 80°C for 15 minutes at a pH in the interval 7-8. As in example 1, pH was adjusted by the addition of 6g of sodium carbonate (2 weight-% of the meal).

Table 2:

Parameter	Ratio - 1 : 20	Ratio - 1 : 10	Ratio - 1 : 5
Weight of shea meal in g	300	300	300
Volume of water in ml	6000	3000	1500
Yield of extract in g	3170	1266	197
Dry matter in extract in % by weight	2.1	3.0	5.7
Extracted dry matter in g	66.6	38.0	11.2
Yield of dry matter in % by weight of meal	22.2	12.7	3.7

According to table 2, the dry matter yield is strongly dependent on the extraction ratio. A ratio in the range of 1:4 to 1:30 will yield crude extracts

with contents in the range from 6 to 1.5% by weight of dry matter. The ratio used in an actual production procedure is dependent on the intended use of the extract (e.g. as is or for further purification) as well as the intended use of the extracted meal residue (e.g. for feedstuffs or for biogas production).

EXAMPLE 6

Shea extract modified by hydrolysis

5% sulphuric acid by weight was added to SHEX-14 extract and the mixture was subsequently hydrolysed for 18 hours at 95°C. The precipitate was washed with alcohol and dried. The resulting sapogenin enriched extract appeared as a dark coloured, waxy substance in a yield of 5 % by weight of the dry matter of SHEX-14.

EXAMPLE 7

Tablet containing hydrolysed shea extract

100 mg tablets containing 20 mg hydrolysed extract from example 6 were formulated as follows:

Active compound:	SHEX-hydrolysed	20 mg
Excipients:	Maize starch	40 mg
	Lactose	26 mg
	Polyvinylpyrrolidone	5 mg
	Silica powder	3 mg
	Carboxymethyl starch	3 mg
	Magnesium stearate	2 mg
	Talc	1 mg